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RESEARCH ARTICLE

# Exploring agronomic traits and breeding prospects of primary tritipyrum and triticale lines to increase grain yield potential

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## Abstract

A study was conducted to evaluate 13 non-Iranian primary tritipyrum ($AABBE^bE^b$, 2n=6x=42) as new amphiploid cereal derived from the hybridization of wild sand couch grass species, *Thinopyrum bessarabicum* (2n=4x=14, $E^bE^b$) and durum (*Triticum durum*, 2n=4x=28, AABB) wheat and five promising triticales for 31 different phenological and agronomic traits in a randomized complete block design (RCBD) with three replications under field conditions. The analysis of variance (ANOVA) showed significant differences between genotypes for most of the studied traits. The factor analysis results revealed five factors that almost explained 89% of the total variance. The stepwise regression and path coefficient analyses revealed that the number of grains per spike and harvest index were the most important traits contributing to the high-yield of the genotypes. Cluster analysis grouped 18 lines into three diverse genotypic clusters. Cluster III included 4115, 4116, and M45 triticale lines indicating the highest value for harvest index as well as grain yield and its components. The non-Iranian primary combined tritipyrum line, (Ka/b)(Cr/b)-5 and promising triticale line No. 4116, showed the highest values for grain-related traits, and therefore these lines may be used in further breeding programs to develop new commercial cereal hybrid/cultivars. Overall, the results indicated grain number per spike and harvest index could be utilized as the key selection criteria in breeding for improvement of grain yield in primary tritipyrum and triticale genotypes.

**Keywords:** Primary tritipyrum, triticale, factor analysis, stepwise regression, path-coefficient analysis, cluster analysis

## Introduction

The escalating global food demand, coupled with the mounting challenges posed by climate change and limited arable land, necessitate a re-evaluation of traditional crop improvement strategies. Tritipyrum, a hybrid between wheat and a wild grass [*Thinopyrum bessarabicum (*Savul. & Rayss) A. Love], and triticale, a man-made hybrid between wheat and rye (*Secale cereal* L.), embody unique genetic resources that have the potential to contribute novel traits for crop improvement under adverse environmental conditions. These intergeneric hybrids may possess the genetic potential to combine the desirable traits of parental species, thereby presenting an opportunity to address the challenges posed by population growth, changing climate patterns, and depleting arable land. The successful exploitation of these resources necessitates a comprehensive understanding of the extent and nature of genetic diversity present within their primary lines. Genetic variation the cornerstone of any breeding effort that offers the potential to introduce valuable attributes such as disease resistance, abiotic stress tolerance, and improved nutritional profiles (Ayalew et al. 2018; Sirakov et al. 2021; Chernook et al. 2022; Farokhzadeh et al. 2022a). Moreover, harnessing

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the diversity present within primary tritipyrum and triticale lines can expedite the development of superior cultivars with tailored characteristics to meet evolving agricultural and consumer demands.

Salinity is one of the major factors that reduces crop yield in many parts of the world (Behera and Hembram 2021). In Iran, around two-thirds of the land is affected by saline soils (Alavi et al. 2021). Non-Iranian primary tritipyrum lines, as a new hexaploid (6x) cereal, has been produced from crosses between durum wheat (*Triticum durum*, 2n= 4x=28, AABB) as the female parent and a brackish sand couch grass specie (*Thinopyrum bessarabicum*, 2n=2x=14, $E^bE^b$) as the male parent since 1997. Although the early generations of this non-Iranian primary tritipyrum were similar in its morphology, considerable fertility, grain set, and salinity tolerance (250 mM NaCl), but showed a few undesirable agronomic and cytogenetic characteristics, including brittle rachis, late maturity, and low meiotic instability (King et al. 1997; Shahsavand Hassani et al. 2000; 2006; Farokhzadeh et al. 2022a). After two decades of cultivation and breeding, efforts to develop a new successful commercial salt-tolerant crop are inevitable (Pirsalami et al. 2021). Many of the problems associated with the cultivation of tritipyrum, which were similar to those initially encountered in the early decades of triticale, were overcome through continuous selection and breeding (Shahsavand Hassani et al. 2021).

To address the issue of aneuploidy in primary tritipyrum, backcrossing primary tritipyrum lines with bread wheat genotypes has resulted in more stable offspring with decreasing aneuploidy rates over multiple generations (Pourfereidouni et al. 2012). Similarly, the creation of secondary triticale lines with high grain numbers and yields from the crossing between hexaploid bread wheat and rye has also been achieved through the production of such lines in triticale (Poehlman 2013). Rezaei et al. (2021) compared the morphophonological characteristics of primary tritipyrum lines with bread wheat and triticale in a dry climate. They found a significant difference between all studied genotypes in terms of various traits, indicating a high genotypic diversity and comparable vegetative growth potential of tritipyrum lines with wheat cultivars and triticale lines.

Grain yield is a polygenic trait. Therefore, breeding genotypes based exclusively on grain yield is not very effective. To enhance breeding efficiency, it is crucial to identify the traits that contribute to the final yield (Dragov et al. 2022). In triticale *and* tritipyrum, understanding and determining the relationships of yield-related traits is very essential for progress in breeding programs. Multivariate analysis methods such as factor analysis, path-coefficient analysis, regression analysis and cluster analysis, can be used to understand the fundamental concepts of multivariate data, biological and functional linkages between traits, describe the correlation among many variables and group different genotypes into distinct groups. These methods have been used to understand the relationship between yield-related traits and to determine the role of these components as indicators to increase performance in breeding of many crops (Hasanuzzaman et al. 2020; Oliveria et al. 2021; Farokhzadeh et al. 2022b; Neykov et al. 2022). Rezaei et al. (2021) studied 14 morphophenological traits in 25 tritipyrum, triticale, and bread wheat genotypes using principal component analysis and extracted six factors that explained 97% of total variance. Regression analysis estimates the value of a quantitative variable concerning its relationship with one or several other quantitative variables. This equation predicts other changes using a variable. Stepwise regression has been utilized to determine the role of yield components and to increase choice efficiency by some traits as effective indicators for breeding purposes (Elmassry and Shal 2020; Hasanuzzaman et al. 2020). The path-coefficient analysis is one of the powerful procedures for examining the relationships between quantitative traits. As a follow-up to correlation analysis, path analysis can improve the precision of selection criteria (Dimitrova-Doneva and Gocheva 2018; Neykov et al. 2022). In a breeding program for complex traits such as grain yield, for which direct selection is not effective, it is necessary to calculate the contribution of each component variable in the observed correlation and divide the correlation into direct and indirect effect components (Hailu et al. 2016). Majumder et al. (2008) using path analysis on spring wheat cultivars, found that spikes per plant, grain per spike, 100- grain weight, and harvest index had a highly positive direct effect on grain yield and concluded that selecting these traits can be effective in improving the grain yield of wheat. Cluster analysis (CA) is an effective multivariate method to evaluate the similarity between genotypes in a group. This method can categorize genotypes and distinguish the best group (Noerwijati et al. 2021). Using cluster analysis, Farokhzadeh et al. (2022b) created three distinct groups of ten wheat and triticale genotypes. Both triticale and tritipyrum are synthetic hexaploid cereals derived from tetraploid wheat as the female parent. Therefore, triticale could be a suitable candidate to evaluate the agronomic potential of tritipyrum lines. Given the above, the present study was conducted to evaluate 13 primary tritipyrum and five promising triticale lines for assessing genetic variation, to understand the interrelationship between the traits and investigate the direct and indirect effects of agronomic traits on grain yield. Classification of genotypes was also carried out based on their genetic capacity and selecting suitable parents with desirable traits for creating new commercial hybrid varieties in breeding programs.

## Materials and methods

### *Plant materials and experimentation*

The material used in the study comprised of six non-

Iranian primary tritipyrum (AABBE$^b$E$^b$, 2n=6x=42) namely, Aziziah/*Thinopyrum bessarabicum*=Az/b, Karim/*Thinopyrum bessarabicum*=Ka/b, Langdon/*Thinopyrum bessarabicum*=La/b, Stewart/*Thinopyrum bessarabicum*=St/b, Creso/*Thinopyrum bessarabicum*=Cr/b, and Langdon(4B/4D)/*Thinopyrum bessarabicum*=La (4B/4D)/b}, seven combined primary tritipyrum lines viz., Macoun/*Th. Bessarabicum* × Creso/*Th. bessarabicum*=(Ma/b)(Cr/b)-3, (Ma/b)(Cr/b)-4, Karim/*Th. bessarabicum*×Creso/*Th. Bessarabicum*=(Ka/b)(Cr/b)-2, (Ka/b)(Cr/b)-3, Ka/b)(Cr/b)-5, (Ka/b)(Cr/b)-6, and Stewart/*Th.bessarabicum*×Creso/*Th.bessarabicum*=(St/b)(Cr/b)-4}, and five promising triticale (AABBRR, 2n=6x=42) lines, such as 4103, 4108, 4115, 4116, and M45 were evaluated in a randomized complete block design (RCBD) with three replications at the research field of Darab University (in a hot and dry climate), Fars, Iran, during 2021-2022 cropping season. Each plot consisted of four rows with 3 m length and 50 cm spacing between the rows. Each plot was 6 m$^2$ and sown with a density of 120 seeds per m$^2$. All the necessary agronomic practices such as irrigation, weed control and other periodic monitoring were performed routinely during the crop season.

### *Observations recorded*

Ten randomly selected plants, grown in the middle rows of each plot, were used for data collection in order to mitigate the effects of neighboring lines on light, water, and essential resources necessary for canopy growth. The data on 31 agronomic traits were recorded as follows: Days to heading (DHE) were calculated when 50% of spikes emerged from the flag leaf sheath, days to physiological maturity (DPM) were calculated when 50% of the spikes in the plot showed a total loss of green colour, plant number per plot (PNP, no./6m$^2$), tiller number per plant (TNP), plant height (PHE) was measured as the distance (cm) from the ground level to the tip of the main spike (excluding the awns), leaf number (LN), node number (NN), spike length (SL, cm) was measured from the base of the rachis to the tip of the terminal spikelet, excluding the awns of the main spikes, spike diameter (SD, cm) was measured using a digital caliper on main spikes after harvest, total spike weight per plant (TSW, g) was calculated from the combined weight of all the spikes of the plants after harvest, fertile spike number per plant (SNP), spike weight mean (SWM, g) was calculated as the ratio of TSW to the SNP, awn length (AL, cm) was measured on the main spikes, internode distance (ID, cm) was defined as the distance between two nodes on the main stem, penultimate leaf length (PLL, cm) and flag leaf length (FLL, cm) were measured as the distance from the base of the ligula to the tip of the leaf. Penultimate main leaf width (PLW, cm) and flag leaf width (FLW, cm) were measured from the widest part of the leaf. Flag leaf sheath length (SHFLL, cm) was measured from the main stem's node to the ligule of the flag leaf from the top at the flowering stage, grain number per main spike (GNS), spikelet number per main spike (SNS), grain mean per spikelet (GMS) was calculated as the ratio of GNS to SNS for the main spikes, grain length (GL, cm) and grain width (GW, cm) were measured based on the averages of 10 healthy and randomly selected central grains within the floret using a digital caliper after harvest, 1000-grain weight (TGW, g), stem weight (SW, g) was determined by harvesting all the stems of plants from the ground level up to the beginning of the spikes in the two middle rows of each plot, and then weighing all the stems, fertility (FE, %) was calculated for 10 randomly selected main spikes using the formula: (number of fertile spikelets / total number of spikelets) × 100, Grain yield (GY, t/ha) was estimated by weighing the grains of harvested plants in each plot (g.m$^{-2}$) when the grains were dry, with a moisture level of about 4%–5%, and then converting the weight to t/ha, biological yield per plot (BY, g/6m$^2$) was measured by weighting the total dry matter produced by plant in each plot, straw yield/plot (SY, g/6m$^2$) was estimated as the difference between grain yield (GY) and biological yield (BY) in each plot, and Harvest index (HI, %) was calculated using the equation, HI = (GY / BY) × 100.

### *Statistical analysis*

Before the analysis of variance, the Shapiro-Wilk test, as a statistical test, was used to investigate the normal distribution of variables using SAS software (version 9.2). Variance analysis of simple and orthogonal contrast was performed on triticale and tritipyrum lines using SAS software (version 9.2). Factor analysis was carried out using principal component analysis with a varimax rotation using SAS (version 9.2). After the varimax rotation of the factors, variables were assigned to independent and various factors according to the factor coefficient. Multivariate linear regression (stepwise) was used to identify the most effective traits on grain yield in the regression model. The multivariate linear regression method satisfied the collinearity and multicollinearity conditions between variables, the normal distribution of the residuals, and independence. A multicollinearity test was performed to test regression hypotheses via the calculation of two statistics, including variance inflation factor (VIF) and tolerance (TOL), using SPSS software (version 24). The VIF and TOL indices were smaller than 10 and greater than 0.1, respectively, which indicated the absence of multicollinearity between variables. Additionally, the Durbin Watson (DW) index, as a test statistic used to detect auto-correlation in the residuals from a statistical model or regression analysis, was performed using SPPS software. If Durbin-Watson statistic is close to 2, it indicates the absence of auto-correlation or serial correlation between the residuals, ensures the independence of the residuals in the regression model (Behpouri et al. 2023). Path analysis was performed to analyze traits' direct and indirect effects on grain yield

**Table 1.** Simple analysis of variance for phenological and agronomic traits in non-Iranian primary and combined primary tritipyrum lines and promising triticale lines

| Source of variation | | Mean squares | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | DF | SNS | SNP | TNP | PNP | LN | NN | SL | SD |
| Replication | 2 | 21.66$^{n.s}$ | 48.92$^{*}$ | 42.06$^{n.s}$ | 311.1$^{*}$ | 0.50$^{n.s}$ | 0. 6$^{n.s}$ | 6.05$^{n.s}$ | 0.01$^{*}$ |
| Lines | 17 | 91.18$^{**}$ | 48.61$^{**}$ | 73.50$^{**}$ | 766$^{**}$ | 0.93$^{**}$ | 0.87$^{**}$ | 8.43$^{**}$ | 0.12$^{**}$ |
| Error | 34 | 3.26 | 11.31 | 14.69 | 76.44 | 0.17 | 0.19 | 2.10 | 0.004 |
| CV (%) | | 10.41 | 19.73 | 20.36 | 8.90 | 8.20 | 10.77 | 11.90 | 8.78 |
| | | GL | GW | AL | HI | BY | GY | SY | SW |
| Replication | 2 | 0.001$^{n.s}$ | 0.00$^{n.s}$ | 0.24$^{n.s}$ | 171.45$^{n.s}$ | 131943$^{n.s}$ | 0.05$^{n.s}$ | 143162$^{n.s}$ | 3696$^{n.s}$ |
| Lines | 17 | 0.01$^{**}$ | 0.00$^{**}$ | 17.07$^{**}$ | 500$^{**}$ | 252919$^{**}$ | 14.45$^{**}$ | 101129$^{*}$ | 129458$^{**}$ |
| Error | 34 | 0.002 | 0.00 | 0.57 | 71.02 | 80432.03 | 0.29 | 49311 | 5001.83 |
| CV (%) | - | 5.34 | 9.09 | 21.19 | 24.29 | 20.53 | 11.52 | 24.58 | 11.67 |
| | | TGW | TSW | SWM | GMS | GNS | ID | PHE | FLL |
| Replication | 2 | 15.98$^{n.s}$ | 20.92$^{n.s}$ | 0.17$^{n.s}$ | 0.15$^{n.s}$ | 37.79$^{n.s}$ | 4.76$^{n.s}$ | 71.31$^{n.s}$ | 123$^{**}$ |
| Lines | 17 | 223$^{**}$ | 234$^{**}$ | 3.55$^{**}$ | 0.31$^{n.s}$ | 852$^{**}$ | 26.83$^{**}$ | 752$^{**}$ | 14.27$^{n.s}$ |
| Error | 34 | 5.23 | 21.66 | 0.24 | 0.16 | 33.26 | 3.89 | 61.98 | 14.09 |
| CV (%) | - | 6.83 | 15.37 | 24.41 | 15.95 | 12.89 | 13.46 | 9.27 | 20.91 |
| | | FLW | SHFLL | PLL | PLW | FE | DHE | DPM | |
| Replication | 2 | 0.15$^{**}$ | 40.85$^{n.s}$ | 113$^{**}$ | 0.20$^{**}$ | 109$^{n.s}$ | 15.40$^{**}$ | 5.68$^{n.s}$ | |
| Lines | 17 | 0.03$^{*}$ | 45.78$^{**}$ | 11.65$^{n.s}$ | 0.03$^{*}$ | 114$^{*}$ | 858$^{**}$ | 493$^{**}$ | |
| Error | 34 | 0.01 | 15.03 | 8.50 | 0.01 | 54.74 | 0.77 | 4.23 | |
| CV (%) | - | 12.03 | 20.46 | 14.23 | 12.60 | 8.06 | 0.63 | 1.12 | |

$^{*}$, $^{**}$ and $^{ns}$: Significant (α = 5%), highly significant (α = 1%) and non-significant, respectively. SNS = Spikelet number per spike, SNP = Spike number per plant, TNP = Tiller number per plant, PNP = Plant number per plot (no./$6m^2$), LN = Leaf number, NN = Node number, SL = Spike length (cm), SD = Spike diameter (cm), GL = Grain length (cm), GW = Grain width (cm), AL = Awn length (cm), HI = Harvest index (%), BY = Biological yield/plot (g/$6m^2$), GY = Grain yield (t/ha), SY= Straw yield/plot (g/$6m^2$), SW = Stem weight (g), TGW = 1000-grain weight (g), TSW = Total spike weight (g), SWM = Spike weight mean (g), GMS = Grain mean per spikelet, GNS = Grain number per spike, ID = Internode distance (cm), PHE = Plant height (cm), FLL = Flag leaf length (cm), FLW = Flag leaf width (cm), SHFLL = Flag leaf sheath length (cm), PLL = Penultimate leaf length (cm), PLW = Penultimateleaf width (cm), FE = Fertility (%), DHE = Days to heading, and DPM = Days to physiological maturity

using the PATH2 program. Cluster analysis was also used to group the genotypes based on the similarity percentage and related traits, using Ward's method by SAS (version 9.2).

## Results and discussion

According to the Shapiro-Wilk test, all variables had a normal distribution. The results of a simple analysis of variance (ANOVA) indicated a significant difference ($P < 0.05$) between the lines for all traits except GMS, FLL and MLL (Table 1). These findings suggest high variation among all lines and are consistent with the findings of Farokhzadeh et al. (2013).

A variance analysis of orthogonal contrast revealed significant differences among the triticale and tritipyrum groups for all phenological and agronomic traits except SW, GMS, FLL and PLL (Table 2). Genetic variation between lines is desirable for effective selection in breeding programs (Silva-Perez et al. 2020; Shahsavand Hassani et al. 2021). The inherent diversity among lines serves as a reservoir of potential traits that can be harnessed through selective processes. The greater the genetic variation, the wider the spectrum of phenotypic attributes available for scrutiny and subsequent enhancement.

The current study employed factor analysis as a powerful tool to discern the latent factors that significantly influence grain yield in tritipyrum and triticale lines. The factor analysis results on 32 different traits identified five factors, each contributing to 54.90, 18.23, 7.99, 4.73, and 3.79% of the total variance, respectively (Table 3). In the first factor, identified as the grain yield-affecting factor, the traits of SWM, SNS, GNS, SD, TGW, GW, AL, GY, and BY had the highest positive impact on the performance of all tritipyrum and triticale lines with loads of 0.97, 0.97, 0.96, 0.95, 0.94, 0.94, 0.92, 0.91, 0.83, and 0.83, respectively. These results indicate that genotypes with high levels of the first-factor score will exhibit higher yield.

**Table 2.** Variance analysis of orthogonal contrast for different traits of tritipyrum and triticale lines

| Source of variation | DF | Mean squares | | | | |
|---|---|---|---|---|---|---|
| | | HI | BY | GY | SY | SW |
| Tritipyrum × Triricale | 1 | 1624$^{**}$ | 2629126$^{**}$ | 159$^{**}$ | 232208$^{*}$ | 16705$^{n.s}$ |
| | | TGW | TSW | GMS | GNS | SNS |
| Tritipyrum × Triricale | 1 | 3372$^{**}$ | 1215$^{**}$ | 0.67$^{n.s}$ | 13111$^{**}$ | 1401$^{**}$ |
| | | SNP | TNP | LN | SD | GL |
| Tritipyrum × Triricale | 1 | 554$^{**}$ | 976$^{**}$ | 9.12$^{**}$ | 1.90$^{**}$ | 0.12$^{**}$ |
| | | AL | ID | PHE | FLL | FLW |
| Tritipyrum × Triricale | 1 | 260$^{**}$ | 139$^{**}$ | 3640$^{**}$ | 0.005$^{n.s}$ | 0.28$^{**}$ |
| | | SHFLL | PLL | NN | DHE | DPM |
| Tritipyrum × Triricale | 1 | 139$^{**}$ | 1.77$^{n.s}$ | 8.82$^{**}$ | 14564$^{**}$ | 8136$^{**}$ |

$^{*}$, $^{**}$ and $^{ns}$: Significant (α= 5%), highly significant (α= 1%) and non-significant, respectively

Therefore, selecting these traits in tritipyrum genotypes leads to increased yield and improved components. Therefore, a strategic focus on these traits during selection and breeding efforts is poised to yield a tangible increase in productivity and enhancements in the fundamental components of yield. These findings are consistent with the study of Moetamadipoor (2015) on wheat and the results reported by Frih et al. (2021), who identified the first two PCs as related to yield components.

The FLL, PLL, SW, SHFLL, and PHE traits with values of 0.87, 0.8, 0.73, 0.67, and 0.63, respectively, had the highest positive coefficients in the second factor. Moreover, SL and SY traits with coefficients of 0.55 and 0.54 were considered as the quadratic coefficient naming elongation components factor. Although the first factor explained most of the variation, traits with the highest factor coefficients in this factor can also be used to select the best genotypes and lines. Figs. 1 and 2 display the 3-D scatter plot and cluster density profile in the factor analysis according to the first and second factors. The third factor, which can be referred to as plant leaf growth, was influenced most heavily by the HI, SL, PLL, and PLW traits, with coefficients of 0.54, 0.47, 0.41, and 0.4, respectively. The fourth factor, referred to as the residual factor, included the SY and SW traits, had coefficients of 0.49 and 0.49, respectively. The fifth factor was most heavily influenced by the SY, BY, and PNP traits with the highest positive coefficients. Additionally, the communality, which represents the percentage of variance in a specific trait accounted for by all the factors (Wedel and Shi 2010), ranged from 0.731 to 0.980 (Table 3). This range shows that the five selected factors captured variability from 73.1% to 98% in all phenological and agronomical traits.

This finding is consistent with the results of Farid et al. (2020), who found that the five extracted factors explained 90% of the total data variation in wheat. Pourfereidouni et al. (2012) studied tritipyrum lines using factor analysis and extracted three factors that explained 68.61% of the total variance. They indicated that the first factor was strongly associated with LN, SL, ID, PHE, FLL, AL, and SNS. Factor 2 was related to TNP, FLW, SNP, and PLL with positive loadings, and the third factor was composed of FLW, SL, and SD with positive factor loadings.

Stepwise multiple regression analysis was used to determine the most powerful interpretive traits that contribute to the most variability in the intriguing variable. Based on the stepwise regression analysis (Table 4), the GNS (0.80) and HI (0.14) traits were included in the model and showed the largest contribution to explaining the yield variation. This finding was consistent with the high correlation coefficients observed between these two traits and grain yield. According to Eid's (2020) results, 90.2% of the total variation in grain yield/plant in wheat could be explained by TGW, GNS, SNP, SNS, and SL variation.

Coefficient of determination ($R^2$) is one of the criteria for determining the accuracy of the model and indicates how much independent variables can explain dependent variable variation. In this study, the final model's correlation

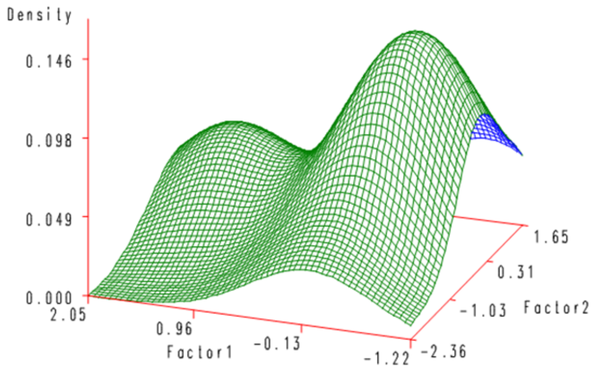

**Fig. 1.** 3-D scatter plot and cluster density for tritipyrum and triticale lines on the basis of first and second factors in principal factor

**Table 3.** Factor loadings and communality of 32 phenological and agronomical traits in tritipyrum and triticale lines

| Traits | Factor 1 | Factor 2 | Factor 3 | Factor 4 | Factor 5 | Communality |
|---|---|---|---|---|---|---|
| Harvest index (HI, %) | 0.448 | -0.640 | 0.535 | -0.012 | -0.168 | 0.925 |
| Biological yield/plot (BY, g/6m$^2$) | 0.831 | 0.254 | -0.094 | 0.253 | 0.362 | 0.959 |
| Grain yield (GY, t/ha) | 0.838 | -0.354 | 0.320 | 0.094 | 0.062 | 0.943 |
| Straw yield/plot (SY, g/6m$^2$) | 0.477 | 0.540 | -0.068 | 0.491 | 0.369 | 0.902 |
| Stem weight (SW, g) | 0.126 | 0.729 | -0.252 | 0.489 | 0.011 | 0.850 |
| 1000-grain weight (TGW, g) | 0.948 | -0.156 | -0.052 | -0.071 | -0.035 | 0.933 |
| Total spike weight (TSW, g) | 0.607 | -0.415 | 0.383 | 0.352 | -0.036 | 0.813 |
| Spike weight mean (SWM, g) | 0.973 | -0.164 | 0.035 | 0.038 | -0.007 | 0.977 |
| Grain mean per spikelet (GMS) | 0.358 | -0.717 | 0.191 | 0.261 | 0.268 | 0.818 |
| Grain number per spike(GNS) | 0.966 | -0.136 | 0.108 | 0.020 | 0.089 | 0.971 |
| Spikelet number per spike (SNS) | 0.971 | 0.146 | 0.049 | -0.121 | -0.006 | 0.980 |
| Spike number per plant (SNP) | -0.810 | -0.046 | 0.329 | 0.245 | 0.260 | 0.894 |
| Tiller number per plant (TNP) | -0.883 | -0.098 | 0.223 | 0.236 | 0.135 | 0.914 |
| Plant number per plot (PNP, no./6m$^2$) | -0.266 | 0.395 | -0.735 | -0.227 | 0.312 | 0.917 |
| Leaf number (LN) | 0.770 | -0.032 | -0.419 | 0.170 | -0.205 | 0.839 |
| Node number (NN) | 0.773 | -0.068 | -0.425 | 0.172 | -0.141 | 0.832 |
| Spike length (SL, cm) | 0.528 | 0.553 | 0.466 | 0.026 | -0.027 | 0.803 |
| Spike diameter (SD, cm) | 0.955 | -0.205 | -0.013 | 0.003 | 0.089 | 0.962 |
| Grain length (GL, cm) | -0.732 | 0.376 | 0.344 | 0.062 | -0.243 | 0.858 |
| Grain width (GW, cm) | 0.924 | -0.181 | -0.100 | 0.063 | 0.016 | 0.901 |
| Awn length (AL, cm) | 0.919 | -0.220 | -0.097 | -0.099 | 0.040 | 0.914 |
| Internode distance (ID, cm) | 0.663 | 0.343 | 0.002 | 0.286 | -0.488 | 0.877 |
| Plant height (PHE, cm) | 0.638 | 0.624 | -0.071 | 0.197 | -0.343 | 0.957 |
| Flag leaf length (FLL, cm) | 0.067 | 0.879 | 0.197 | -0.191 | 0.115 | 0.866 |
| Flag leaf width (FLW, cm) | 0.714 | 0.461 | 0.181 | -0.370 | 0.144 | 0.912 |
| Flag leaf sheath length (SHFLL, cm) | 0.509 | 0.664 | 0.076 | -0.029 | -0.154 | 0.731 |
| Penultimate leaf length (PLL, cm) | -0.037 | 0.796 | 0.414 | 0.093 | 0.133 | 0.833 |
| Penultimate leaf width (PLW, cm) | 0.608 | 0.491 | 0.402 | -0.382 | -0.016 | 0.919 |
| Fertility (FE, %) | 0.800 | 0.194 | 0.213 | -0.064 | 0.264 | 0.797 |
| Days to heading (DHE) | -0.972 | 0.085 | 0.104 | 0.083 | -0.050 | 0.973 |
| Days to physiological maturity (DPM) | -0.955 | 0.123 | 0.068 | 0.173 | -0.106 | 0.973 |
| Eigen value | 17.57 | 5.83 | 2.56 | 1.51 | 1.21 | |
| Proportional variance (%) | 54.90 | 18.23 | 7.99 | 4.73 | 3.79 | |
| Cumulative variance (%) | 54.90 | 73.13 | 81.12 | 85.84 | 89.63 | |

coefficient ($R^2$) was 0.95, which can explain 95% of yield variation (Table 4).

Golparvar et al. (2002) showed that seven agronomic traits of wheat, including BY, HI, GY per spike, grain number per plant, GNS, grain weight, and PHE, verified 98.4% of GY variation. In the Soleimani Fard and Naseri (2020) study, BY, HI, and TGW accounted for 98% of the variation in wheat GY in the corresponding regression model.

The results of the final model of stepwise regression analysis on primary tritipyrum lines (Table 5) indicated that HI (0.82), SNP (0.05), and TGW (0.04) had greater contribution in explaining the 91% of GY variation. Amini et al. (2005)

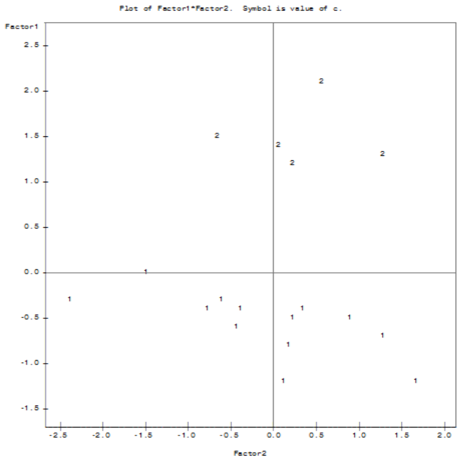

**Fig. 2.** Clustering for tritipyrum and triticale lines on the basis of first and second factors from principal factor analysis of all studied traits

**Table 4.** Stepwise regression analysis of grain yield as dependent and other traits as independent variables for primary tritipyrum and triticale lines

| Traits | F | Partial $R^2$ | $R^2$ | Entering into model, respectively |
|---|---|---|---|---|
| Grain number per spike (GNS) | 62.02 | 0.80 | 0.80 | 1 |
| Harvest index (HI, %) | 40.56 | 0.14 | 0.95 | 2 |

**Table 5.** Stepwise regression analysis of grain yield as dependent and other traits as independent variables for non-Iranian primary and combined primary tritipyrum lines

| Traits | F | Partial $R^2$ | $R^2$ | Entering into model, respectively |
|---|---|---|---|---|
| Harvest index (HI, %) | 53.09 | 0.82 | 0.82 | 1 |
| Spike number per plant (SNP) | 4.35 | 0.05 | 0.88 | 2 |
| 1000-grain weight (TGW, g) | 3.78 | 0.04 | 0.91 | 3 |

**Table 6.** Stepwise regression analysis of grain yield as dependent and other traits as independent variables for promising triticale lines

| Traits | F | Partial $R^2$ | $R^2$ | Entering in to model, respectively |
|---|---|---|---|---|
| Plant number per plot (PNP, no./6m$^2$) | 17.11 | 0.85 | 0.85 | 1 |
| Grain number per spike (GNS) | 5.93 | 0.11 | 0.96 | 2 |
| Total spike weight (TSW, g) | 21.15 | 0.035 | 0.99 | 3 |

concluded that HI and BY traits in a stepwise regression analysis model could explain a high rate of GY variation in wheat.

The $R^2$ coefficient (0.99) in promising triticale lines (Table 6) showed the PNP (0.85), GNS (0.11), and TSW (0.04) had a higher effect on grain yield variation. The results of other studies (Fellahi et al., 2013; Shah et al. 2018; Ullah et al. 2018; Gaikwad et al. 2023), showed the importance of these traits for yield variation in the stepwise regression model in wheat. Correlation between grain yield and yield-associated traits measures reciprocal relations without causation presumption, but the result of path analysis can characterize genotypic correlations to direct and indirect effects (Khan et al. 2020). In this study, results of path analysis (Table 7) showed that the GNS (0.66), and HI (0.44) traits had a high, positive, and significant direct effect on GY. The indirect effect of GNS on GY through HI was 0.31, while the indirect effect of HI on GY through GNS was 0.21. Zafarnaderi et al. (2013) and Ranjbar et al. (2015) confirmed the importance of direct effect of these two traits on wheat GY. However, Patel et al. (2020) reported that the grain weight per main spike, BY, and HI had high and positive direct effects on grain yield per plant in wheat.

Cluster analysis using Ward's method grouped 18 genotypes into three major clusters, as shown in Fig. 3. Cluster I comprised nine tritipyrum lines (50%) followed by cluster II with six tritipyrum and triticale lines (33.33%), and cluster III with three triticale lines (16.67%). Cluster I was divided into two tritipyrum subgroups including: [Az/b, (Ka/b)(Cr/b)-6, La(4B/4D)/b, (Ma/b)(Cr/b)-4, Cr/b, and (St/b)(Cr/b)-4] and [Ka/b, (Ka/b)(Cr/b)-3 and (Ka/b)(Cr/b)-5]. Cluster II comprised two subgroups including: [tritipyrum: La/b, St/b, and (Ma/b)(Cr/b)-3] and [tritipyrum and triticale: (Ka/b)(Cr/b)-2, 4106, and 4108]. Whereas, cluster III comprised only three triticale lines: 4115, 4116, and M45.

Wide variation in cluster means (Table 8) was observed for HI from 28.72% (cluster II) to 42.64% (cluster III), and for SNP from 11.36 (cluster III) to 19.29 (cluster I). Traits of TNP, GL, DHE, and DPM recorded maximum values in cluster I and minimum in cluster III, respectively. On the other hand, Cluster II showed the highest mean for SW, SNP, PNP, FLL, and PLL.

Cluster III had the highest mean for HI, BY, GY, SY, TGW, TSW, SWM, GMS, GNS, SNS, LN, NN, SL, SD, GW, AL, ID, PHE, FLW, SHFLL, PLW, and FE than the other clusters. Cluster III was composed of triticale lines 4115, 4116, and M45, characterized by early flowering and maturity. With the current climate change scenario, where global temperature is increasing, many areas of the world are prone to severe drought. Therefore, developing drought-tolerant crops with a shorter vegetative stage and earlier flowering is a promising strategy (Shavrukov et al. 2017; Azameti et al. 2022; Farokhzadeh et al. 2022b). Early maturing genotypes are crucial in late planting conditions to avoid the harmful effects of high temperatures, particularly during the reproductive phase (Adilova et al.

**Table 7.** Path analysis for different traits of primary tritipyrum and triticale lines

| Direct and indirect effect of traits on grain yield in path coefficient analysis | | | | |
|---|---|---|---|---|
| Traits | Direct effect | Indirect effect via | | Correlation with the grain yield (GY, t/ha) |
| | | Grain number per spike (GNS) | Harvest index (HI, %) | |
| Grain number per spike (GNS) | 0.655** | - | 0.310 | 0.664** |
| Harvest index (HI, %) | 0.444** | 0.210 | - | 0.886** |

**: highly significant (α= 1%)

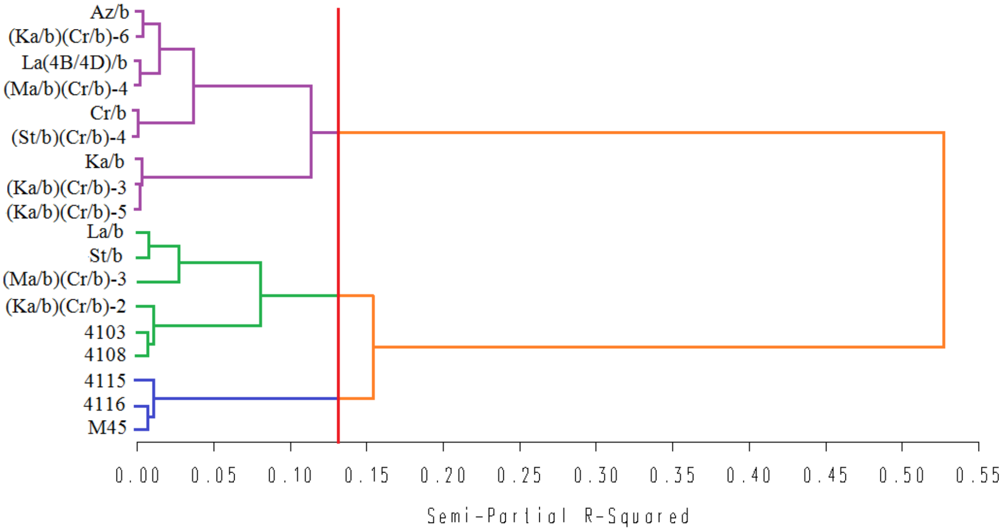

**Fig. 3.** Dendrogram of cluster analysis for different traits in promising triticale and primary tritipyrum lines using Ward's method

2020). Previous researchers have also examined the primary selection of high-yield genotypes using cluster analysis to evaluate their economic traits and adaptation characteristics (Adilova et al. 2020; Farokhzadeh et al. 2022b). In the present study, cluster III, consisting of triticale lines 4115, 4116, and M45, had the highest GY value as well as its components and HI, which can be utilized to improve GY in breeding programs.

These findings suggest that the selection of superior parental lines should be based on a combination of traits as well as differences between clusters to achieve a high level of genetic variation for further improvement (Singh and Panwar 2005; Khan et al. 2020). According to Reynolds et al. (2017), HI is a crucial breeding criterion for enhancing productivity under both normal and stressful conditions, and it should be consistently considered along with an increasing number of grains per m$^2$. Moreover, Neykov et al. (2022) demonstrated that grain mass per spike and the number of spikes per m$^2$ have the highest direct effect on barley grain yield using path-coefficient analysis.

The grain yield, as the most critical economic component, is a result of yield components and other related traits. Identifying these components and their relationship with yield can be impressive in achieving optimal yield. The results of the present study are consistent with other studies (Gaju et al. 2009; Farokhzadeh et al. 2013; Zhang et al. 2016) to distinguish traits that affect GY such as SNP, GNS, and TGW. Many studies have reported GNS as a crucial selection criterion for GY in different crops, including wheat (Dragov et al. 2022; Neykov and Doneva, 2020). Passioura (2020) reported that the GNS was the only "universal" feature that can successfully increase GY via selection, regardless of environmental conditions. Farokhzadeh et al. (2022b) integrated the results of multivariate statistics and supervised learning methods and reported that GNS, SNS, and HI could be used to create a selection index for the high grain yield in bread wheat cultivars and triticale lines. These reports are consistent with the findings of present study. The tritipyrum line (Ka/b)(Cr/b)-5 and the promising triticale line 4116 had the highest TSW, TGW, GNS, GY, and HI values. Additionally, line 4116 had the highest SD, SNP, SNS, and SL values.

The information on the relationship between agronomical traits and grain yield can be useful for breeding programs to increase yield. This study applied factor analysis, stepwise regression, and path analyses to investigate the relationship between grain yield and other phenological and agronomic traits. Based on the multivariate methods

**Table 8.** Traits means of the tritipyrum and triticale genotypes grouped in different clusters

| Traits | Cluster I | Cluster II | Cluster III |
|---|---|---|---|
| Harvest index (HI, %) | 36.03 | 28.72 | 42.64 |
| Biological yield/plot (BY, g/6m$^2$) | 1166.90 | 1467.49 | 1849.51 |
| Grain yield (GY, t/ha) | 3.97 | 4.20 | 7.77 |
| Straw yield/plot (SY, g/6m$^2$) | 786.00 | 965.28 | 1131.71 |
| Stem weight (SW, g) | 460.88 | 814.79 | 623.91 |
| 1000-grain weight (TGW, g) | 28.78 | 32.95 | 48.55 |
| Total spike weight (TSW, g) | 27.29 | 29.72 | 37.46 |
| Spike weight mean (SWM, g) | 1.40 | 1.96 | 3.95 |
| Grain mean per spikelet (GMS) | 2.58 | 2.50 | 2.69 |
| Grain number per spike (GNS) | 35.81 | 45.26 | 70.49 |
| Spikelet number per spike (SNS) | 13.93 | 18.00 | 26.27 |
| Spike number per plant (SNP) | 19.29 | 16.53 | 11.36 |
| Tiller number per plant (TNP) | 21.79 | 18.00 | 11.62 |
| Plant number per plot (PNP, no./6m$^2$) | 96.93 | 104.56 | 89.44 |
| Leaf number (LN) | 4.78 | 5.09 | 5.91 |
| Node number (NN) | 3.80 | 4.09 | 4.91 |
| Spike length (SL, cm) | 11.54 | 12.30 | 13.84 |
| Spike diameter (SD, cm) | 0.68 | 0.81 | 1.12 |
| Grain length (GL, cm) | 0.90 | 0.89 | 0.79 |
| Grain width (GW, cm) | 0.20 | 0.23 | 0.27 |
| Awn length (AL, cm) | 2.26 | 3.57 | 7.61 |
| Internode distance (ID, cm) | 12.57 | 16.61 | 17.10 |
| Plant height (PHE, cm) | 72.98 | 94.37 | 101.61 |
| Flag leaf length (FLL, cm) | 17.49 | 18.62 | 18.01 |
| Flag leaf width (FLW, cm) | 0.99 | 1.07 | 1.14 |
| Sheath flag leaf length (SHFLL, cm) | 17.14 | 20.34 | 21.58 |
| Penultimate leaf length (PLL, cm) | 19.89 | 21.47 | 20.32 |
| Penultimate leaf width (PLW, cm) | 0.94 | 0.99 | 1.08 |
| Fertility (FE, %) | 88.44 | 93.71 | 97.60 |
| days to heading (DHE) | 148.70 | 136.06 | 112.67 |
| days to physiological maturity (DPM) | 189.11 | 181.72 | 162.78 |

used in present study, GNS and HI can be recommended for improving grain yield in non-Iranian tritipyrum and promising triticale lines in future hybridization breeding programs. Among the tritipyrum and triticale lines, (Ka/b)(Cr/b)-5 primary combined line and 4116 promising triticale line with the highest yield showed the best performance for most phenological and agronomic traits.

In conclusion, this study's utilization of multivariate statistical analyses has unveiled the intricate web of relationships between agronomical traits and grain yield in tritipyrum and promising triticale lines. Discovering the relationship between the important agronomic traits can stand as a beacon of promise for application in practical breeding programs to increase grain yield potential in tritipyrum and triticale lines.

## Authors' contributions

Conceptualization of research (SF); Data curation (SF); Formal analysis and software (SF); Investigation (SF, ESM); Methodology (SF); Project administration (SF, HSH, ST), Supervision (SF, ST, ESM); Visualization (SF, ST); Writing – original draft (SF); Writing – review and editing (SF, HSH, ZZ, ST), Revision (SF, ST).

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